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	L4	(adoptive immunotherapy)	1313
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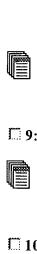
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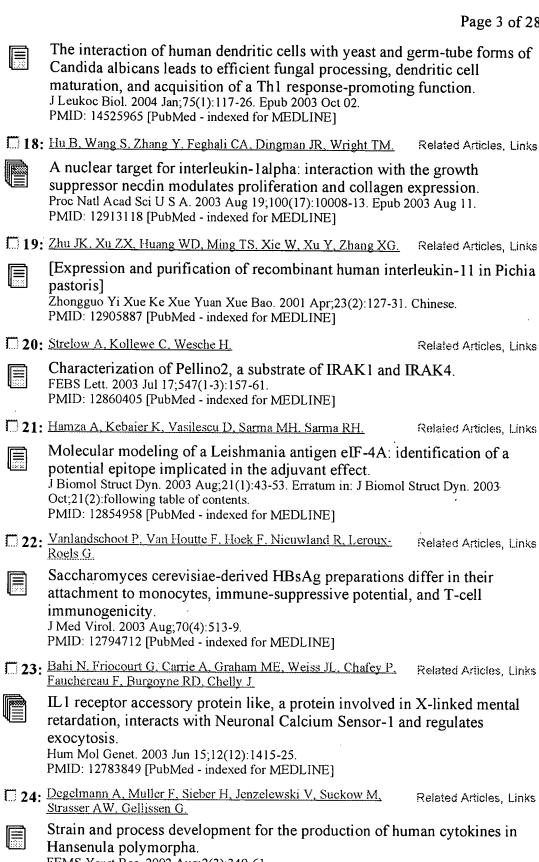
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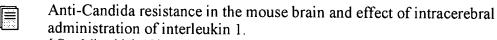
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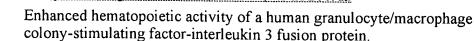


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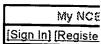
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Secretion of mature mouse interleukin-2 by Saccharomyces cerevisiae: use of a general secretion vector containing promoter and leader sequences of the mating pheromone alpha-factor.

Miyajima A, Bond MW, Otsu K, Arai K, Arai N.

We have constructed a general expression vector which allows the synthesis and secretion of processed gene products in Saccharomyces cerevisiae. This vector contains yeast DNA, including the promoter of the mating pheromone (alpha-factor), its downstream leader sequence, and the TRP5 terminator. A cDNA [encoding mature mouse interleukin-2 (IL-2); Yokota et al., Proc. Natl. Acad. Sci. USA 82 (1984) 68-72] was fused immediately downstream to the alpha-factor leader sequence. The resulting recombinant plasmid directed the synthesis of mature mouse IL-2 in S. cerevisiae, with most of the T-cell growth-factor (TCGF) activity secreted into the culture fluid and extracellular space. TCGF activities in the cell extract, as well as in the culture fluid, increased in parallel with cell growth. Production of mature mouse IL-2 was inhibited by tunicamycin (TM), with precursor molecules accumulating in the cell extract. The precursor was processed accurately at the junction between the alpha-factor peptide leader sequence and the coding sequence downstream, yielding mature IL-2. The Mr of the secreted mouse IL-2 determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was 17 kDal, a value expected for the mature mouse IL-2 polypeptide based on the nucleotide (nt) sequence.

PMID: 3902570 [PubMed - indexed for MEDLINE]

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Molecular cloning and expression of DNA encoding ovine interleukin 2.

Bujdoso R, Williamson M, Roy D, Hunt P, Blacklaws B, Sargan D, McConnell I.

Department of Veterinary Pathology, Summerhall, University of Edinburgh.

We have generated DNA encoding the mature form of ovine interleukin 2 (IL-2) by polymerase chain reaction (PCR) using primers complementary to sequences at the 5' and 3' ends of human, murine and bovine IL-2 cDNA. The predicted PCR product of 400 bp was ligated into the yeast Ty-P1 galactoseinducible expression vector pOGS40 which was used to transform yeast spheroplasts. The fusion protein, with a Factor Xa proteolytic cleavage site between ovine IL-2 and the P1 fusion partner, was expressed from galactoseinduced transformed yeast. P1:IL-2 fusion protein, which self-assembles into virus-like particles (VLPs) due to the interaction of the P1 protein, was purified from lysates of mechanically disrupted yeast by centrifugation on a discontinuous sucrose gradient. Fusion protein was detected in Western blot analysis with polyclonal antisera raised to recombinant bovine IL-2. Soluble recombinant ovine IL-2 was released from the PI fusion protein by cleavage with Factor Xa enzyme. After purification recombinant ovine IL-2 was functionally active as shown by its ability to support the proliferation of Con A-activated T cells and was capable of generating maedi visna virus-specific cytotoxic T cells from primed precursor cells. The availability of recombinant ovine IL-2 will greatly help the analysis of the specificity of pathogen-specific cells in the sheep.

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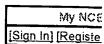
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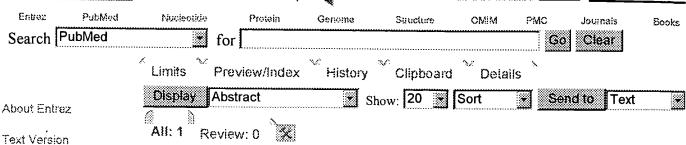
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## FUEL-TEXY ARTICLE

Molecular cloning and expression of DNA encoding ovine interleukin 2.

Bujdoso R, Williamson M, Roy D, Hunt P, Blacklaws B, Sargan D, McConnell I.

Department of Veterinary Pathology, Summerhall, University of Edinburgh.

We have generated DNA encoding the mature form of ovine interleukin 2 (IL-2) by polymerase chain reaction (PCR) using primers complementary to sequences at the 5' and 3' ends of human, murine and bovine IL-2 cDNA. The predicted PCR product of 400 bp was ligated into the yeast Ty-P1 galactoseinducible expression vector pOGS40 which was used to transform yeast spheroplasts. The fusion protein, with a Factor Xa proteolytic cleavage site between ovine IL-2 and the P1 fusion partner, was expressed from galactoseinduced transformed yeast. P1:IL-2 fusion protein, which self-assembles into virus-like particles (VLPs) due to the interaction of the P1 protein, was purified from lysates of mechanically disrupted yeast by centrifugation on a discontinuous sucrose gradient. Fusion protein was detected in Western blot analysis with polyclonal antisera raised to recombinant bovine IL-2. Soluble recombinant ovine IL-2 was released from the P1 fusion protein by cleavage with Factor Xa enzyme. After purification recombinant ovine IL-2 was functionally active as shown by its ability to support the proliferation of Con A-activated T cells and was capable of generating maedi visna virus-specific cytotoxic T cells from primed precursor cells. The availability of recombinant ovine IL-2 will greatly help the analysis of the specificity of pathogen-specific cells in the sheep.

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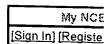
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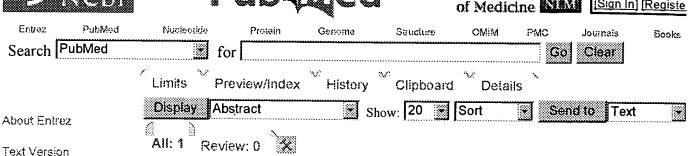
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Effects of yeast expressed recombinant interleukin-2 and interferon-gamma on physiological changes in bovine mammary glands and on bactericidal activity of neutrophils.

Wedlock DN, Doolin EE, Parlane NA, Lacy-Hulbert SJ, Woolford MW, Buddle BM.

AgResearch, Wallaceville Animal Research Centre, Upper Hutt, New Zealand.

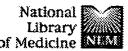
The physiological effects of intramammary infusions of recombinant bovine cytokines in six lactating dairy cows on the quality and yield of milk and the bactericidal activity of milk neutrophils were investigated. Recombinant bovine interleukin-2 (rboIL-2) and interferon-gamma (rboIFN-gamma) were produced in the yeast Pichia pastoris. Two animals were given rboIL-2 (2 x 10 (5) units) in two quarters, two animals were given rboIFN-gamma (6.5 x 10(5) units) in two quarters, and the other two cows received a dose of rboIL-2 in one quarter and rboIFN-gamma in a second quarter. In addition, each animal was given phosphate-buffered saline (PBS) in the other two quarters as a control. Somatic cell counts and conductivity of the fore milk were monitored before and after infusion. Neutrophils were isolated from quarter milk samples 36 h after infusion of cytokine or PBS and their bactericidal activities against Staphylococcus aureus were measured in vitro with a colorimetric assay. Quarters infused with rboIL-2 or rboIFN-gamma showed significant but transitory increases in both milk somatic cell counts and conductivity when compared with preinfusion values and with control quarters. There were minimal effects on daily milk yield. Neutrophils isolated from milk from quarters infused with rboIL-2 showed enhanced bactericidal activity against Staph. aureus. The bacterial killing from rboIL-2 treated quarters was significantly greater, with a mean of 63.5% compared with a mean of 5.4% for neutrophils taken from uninfected quarters to which PBS had been administered. The bactericidal activities for quarters treated with rboIFNgamma and infected quarters treated with PBS were 15.0 and 30.0% respectively. The results indicate that intramammary infusions of rboIL-2 and rboIFN-gamma to lactating cows are well tolerated, and that rboIL-2 can activate milk neutrophils and augment their bactericidal activity.

PMID: 10840672 [PubMed - indexed for MEDLINE]

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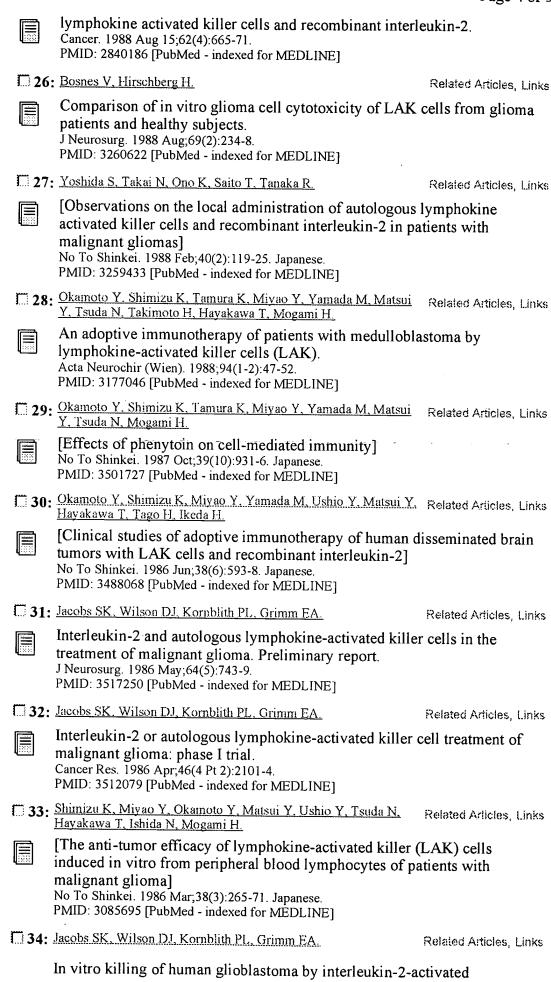
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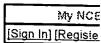
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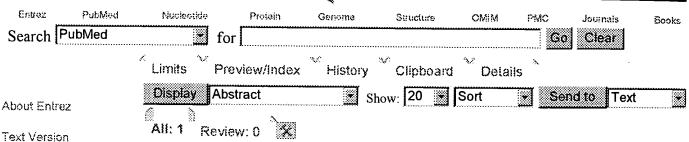
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Interleukin-2-activated lymphocytes from brain tumor patients. A comparison of two preparations generated in vitro.

Kruse CA, Mitchell DH, Lillehei KO, Johnson SD, McCleary EL, Moore GE, Waldrop S, Mierau GW.

Department of Surgery, University of Colorado Health Sciences Center, Denver 80262.

Two preparations of human recombinant interleukin-2 (rIL-2)-activated lymphocytes from patients harboring malignant brain tumors were characterized as autologous-stimulated lymphocytes (ASL) and lymphokineactivated killer (LAK) cells. ASL were generated from Ficoll-Paque-isolated, nonadherent, defibrinated peripheral blood lymphocytes (PBL) that were stimulated overnight with phytohemagglutinin (PHA) and cultured with rIL-2 (100 U/ml) for 10 days. LAK cells were produced by culturing all PBL in rIL-2 (500 U/ml) for 4 days. In 4-hour chromium release assays, LAK cells showed greater cytotoxicity than ASL against natural killer (NK)-sensitive and NK-resistant tumor cell lines; by 18 hours, the effectiveness of ASL equaled that of LAK cells. By electron microscopic study, PBL, LAK cells, and ASL showed differences. The helper/inducer to suppressor/cytotoxic ratio (T4+/T8+) of PBL, LAK cells, and ASL was 1.1:1, 1.0:1, and 0.4:1, respectively. ASL, when compared with PBL or LAK cells, have a significantly higher percentage of MO1+/DR+ and T8+/9.3+ subpopulations. ASL and LAK cells, used for the therapy of gliomas, are distinct.

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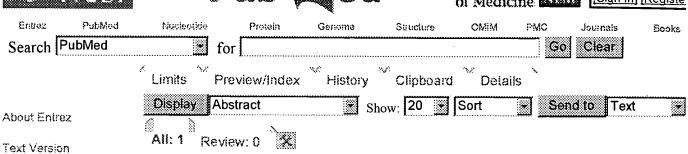
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Adoptive immunotherapy using lymphokine-activated killer (LAK) cells and interleukin-2 for recurrent malignant primary brain tumors.

Sankhla SK, Nadkarni JS, Bhagwati SN.

Department of Neurosurgery, Bombay Hospital, India.

Ten patients with recurrent malignant primary brain neoplasms were treated with adoptive immunotherapy using lymphokine-activated killer (LAK) cells and interleukin-2 (IL-2). Nine patients had supratentorial glioma and they received multiple intratumoral instillations of LAK cells through reservoircatheter system or burrhole. The other patients with disseminated subarachnoid metastases from posterior fossa medulloblastoma received immunotherapy via lumbar subarachnoid route. A partial and transient clinical response was observed in two patients. following the therapy, and a cystic transformation of the essentially solid tumour was noted on the CT scans of these two patients. No significant clinical or radiological response to the treatment was observed in the remaining 8 patients. The results of this preliminary study reveal limitations of the regional intratumoral adoptive immunotherapy using currently available techniques and provide sufficient evidence of its effectiveness to warrant further investigations.

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Key (yoo-kăr'e-ot, -e-ot)

A single-celled or multicellular organism whose cells contain a distinct membrane-bound nucleus.

[eu-+ Greek karuotos, having nuts (from karuon, nut. See kar- in Indo-European Roots).]

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